Looking for Atoms

Designer Enzymes for Industry and Medicine

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Andrey Kovalevsky, postdoctoral fellow at the Los Alamos Protein Crystallography Station (PCS), stares intently at a computer screen filled with dark dots on a white background. It looks like the old photographic plates of stars in the night sky. But Kovalevsky is not looking for astronomical objects. He's trying to infer the positions of atoms in a crystallized enzyme. He hopes to uncover the complete structure and operation of xylose isomerase, an important enzyme that catalyzes the conversion of one type of sugar into another.

Enzymes are large protein molecules made by living organisms. They are the most-powerful catalysts known on Earth, speeding up biochemical reactions that, in their absence, would never occur. How is that possible? At the PCS, Kovalevsky and other young scientists hope to learn the enzymes' secret ways and then re-engineer them to do new tasks or perform old ones more efficiently.

Until the PCS opened 5 years ago at the Los Alamos Neutron Science Center (LANSCE), most scientists used only x-ray crystallography to learn the atomic-level structure of proteins. Researchers would shine an x-ray beam on a crystallized protein and record the diffraction "peaks"—intense spots created when x-rays diffract (scatter coherently) from the crystal's orderly array of atoms. From the pattern and intensity of those peaks, scientists could deduce the three-dimensional arrangement of atoms making up the protein's structure.

An astonishing 60,000 proteins have been analyzed this way, but for enzymes, the resulting structural models have one serious limitation—the hydrogen atoms are missing. X-ray diffraction peaks from hydrogen are usually too faint to see.

Diffraction peaks are created when x-rays scatter from the electron cloud surrounding an atomic nucleus. Hydrogen has only one electron in its cloud, so it scatters x-rays very weakly. With 50 percent of the atoms in an enzyme being hydrogen, x-ray crystallography can provide only an incomplete picture of an enzyme's structure.

Marc-Michael Blum, a German researcher using the PCS, explains, "Some enzymes are like Swiss army knives. Their surfaces look almost alike, but they contain very different tools. Only by knowing the atomic arrangement of those tools, including the positions of the hydrogen atoms, can you figure out how an enzyme really works."

The positions of the hydrogen atoms are especially important because they invariably get shuffled about during enzyme-catalyzed reactions. The hydrogen positions give critical cues about how the reaction moves forward.

"Researchers now have a way around the hydrogen problem," says Paul Langan, team leader of the PCS. "Here at the PCS, they can use neutrons, not x-rays, to do the crystallography and within a few weeks have enough data to locate the hydrogen positions relative to the other atoms in an enzyme." (See "Learn More")

Unlike x-rays, neutrons scatter as strongly from hydrogen as from other elements because they scatter from the atomic nucleus rather than the electron cloud. But the scattering strength depends on the nuclear composition. By replacing a crystal's hydrogen with the isotope deuterium, researchers can add a distinct signal component that helps them locate the positions of the replaced atoms. Conveniently, Mary Jo Waltman, a crackerjack technologist, is available to help PCS users grow deuterated crystals in special deuteration laboratories.

Kovalevsky adds, "If we can crystallize the enzyme at various catalytic stages, we can visualize, step by step, how it changes shape and shuffles hydrogens to different locations." Seeing this level of detail has reveled a big surprise: some enzymes take a much more active role in the reaction chemistry than commonly thought.

That conclusion is based on x-ray structures, the results of biochemical experiments in solution, theoretical calculations, and more. "The neutron work merely ‘dots the i's,'" comments Benno Schoenborn, inventor of neutron protein crystallography. "But that final level of information keeps the theorists honest."

It can also provide a firm basis for engineering an improved version of an enzyme, as a

group of German researchers is showing.

Birth of a New Technique

Amidst all the excitement and bustle at the Los Alamos Protein Crystallography Station (PCS) is an intense, urbane gentleman, Benno Schoenborn, giving very-specific advice on how to get the most out of the instrument he and his colleagues have designed. Schoenborn first dreamt of making neutron crystallography a tool for biology more than 40 years ago. Back then, neutrons were available only at nuclear reactors, and safe reactor operations put a firm lid on neutron intensity (the number of neutrons per second, per square centimeter, coming out of the reactor). Although Schoenborn used reactor neutrons to prove that neutron crystallography would work on proteins, he saw it as a labor of love, taking many, many months to collect enough data for a single protein structure.

A neutron diffraction pattern.

The intensity lid came off with the advent of the spallation neutron source at the Los Alamos Neutron Science Center (LANSCE). A high-intensity pulse of neutrons is produced each time a proton pulse from the LANSCE accelerator hits a tungsten target. Some of those neutrons are directed down an evacuated tube to the crystal mounted in front of the PCS neutron detector.

Benno Schoenborn

Like runners coming out of the gate, the neutrons stretch out according to speed along an evacuated guide tube, the fastest arriving at the crystal sample first and the slowest 10 milliseconds (1/1000th of a second) later. Each neutron’s arrival is like a time stamp that announces its speed, or energy. Neutrons of different energies are diffracted by different sets of planes in the crystal. The PCS records the diffraction patterns created at 100 different energies (times) within the duration of each 10-millisecond pulse.

A neutron pulse strinking and diffreacting from a sample at he PCS.

Schoenborn, with colleagues Eric Pitcher, Phil Ferguson, and later Paul Langan, designed every component of the PCS to maximize the neutron intensity and reduce background scattering so scientists could collect excellent data in 15 to 30 days of beam time using much-smaller crystals. The big payoff is that the larger proteins, for which large crystals are nearly impossible to grow, have become eligible for neutron crystallography.